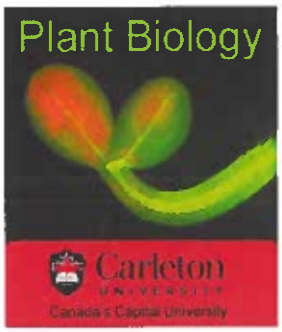

41st Annual
Plant Development Workshop

Carleton University, Ottawa
November 10th, 2007

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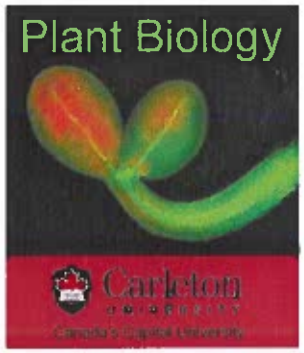


41st Annual Plant Development Workshop

Carleton University, Ottawa
November 10, 2007

Keynote speakers:

Xuemei Chen (UC Riverside)
Role of microRNAs in plant development

Vivian Irish (Yale)
EvoDevo of petal development

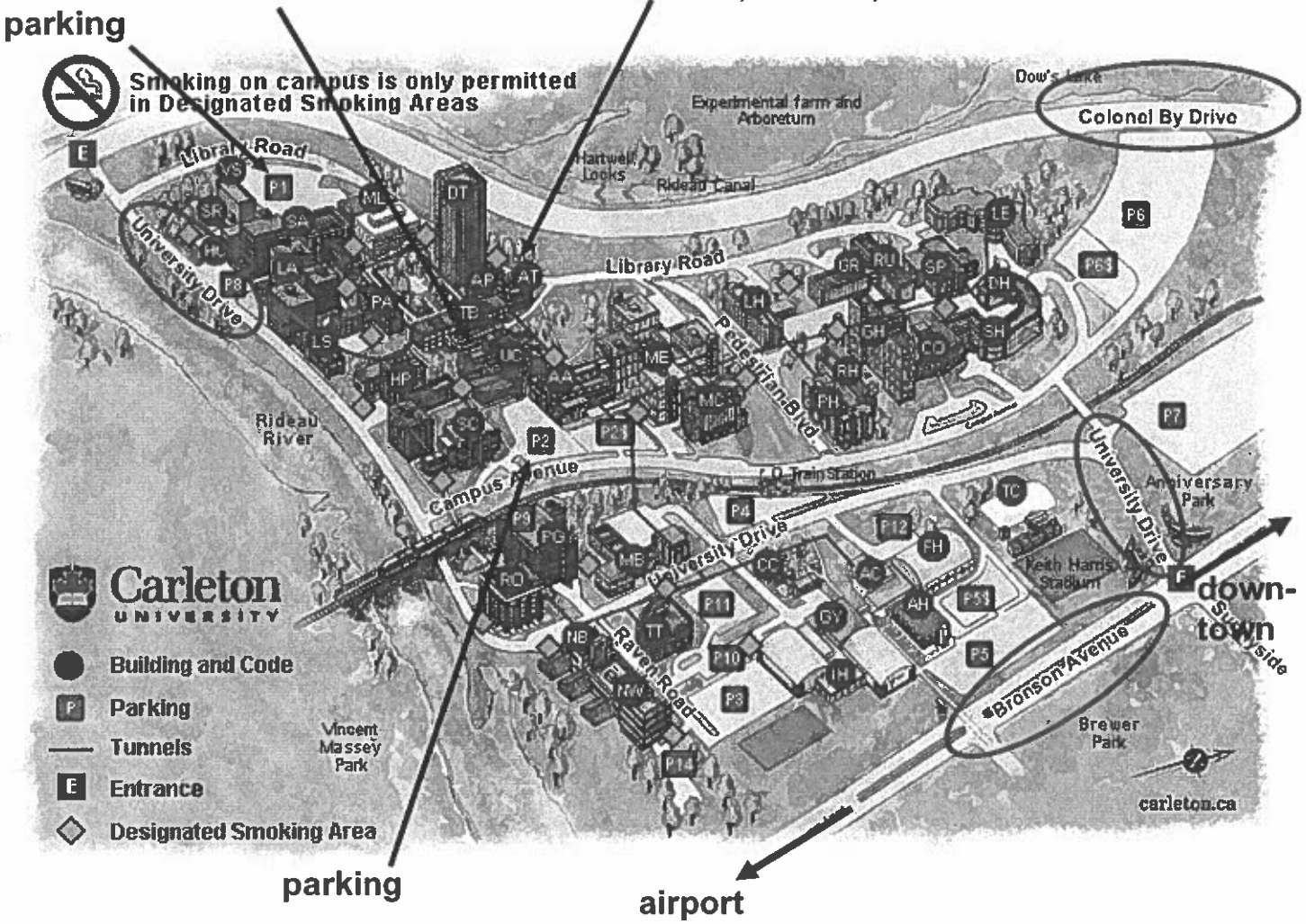
For registration and info contact:
shelley_hepworth@carleton.ca

Carleton University
41st Annual Plant Development Workshop

Parking on campus is free on Saturdays.

Galleria, University Centre
Wine and Cheese Social

Azrieli Theatre, Room 302
Talks, Posters, and Lunch



41st Annual Plant Development Workshop

Saturday November 10th, 2007
Carleton University, 302 Azrieli Theatre

Schedule:

8.00-8.55 Registration/Breakfast/Poster Set-up

8.55-9:00 Welcome: Shelley Hepworth

Session 1 Chair: Tamara Western (McGill University)

9.00-9.50 *Plenary Talk 1:* Xuemei Chen (University of California, Riverside)
miRNA biogenesis and roles of miR172 in flower development

9.50 – 10.10 Vojislava Grbic (University of Western Ontario)
Natural variation in shoot morphology of *Arabidopsis thaliana* caused by *HUA2*

10.10-10.30 Rashida Patel* (University of Toronto, Scarborough)
Characterization of TCP8 and TCP15 genes in *Arabidopsis thaliana*: genes with implications for regulating multiple aspects of plant architecture

10.30-11:00 Coffee Break and **Posters 1-6** (Azrieli Theatre Lobby)

Session 2 Chair: Gopal Subramaniam (ECORC, Agriculture and Agri-Food Canada)

11.00-11.20 Eric Chevalier* (Université de Montréal)
RALF, a secreted peptide involved in plant reproduction, stomatal opening and root formation

11.20-11.40 Donald Smith (Plant Sciences Department, McGill University)
Signals in the Underground: Microbial Signals and Plant Development

11.40-12.00 Andrej Arsovski* (Department of Biology, McGill University)
PATCHY encodes a putative beta-xylosidase/arabinofuranosidase required for pectin sidechain modification in Arabidopsis mucilage secretory cells

12.00-12.20 Xingyun Qi* (Department of Biology, McGill University)
Functional characterization of small Rab-A1 GTPases

12.20-1.30 Lunch and **Posters 7-18** (Azrieli Theatre Lobby)

Session 3 Chair: Shelley Hepworth (Carleton University)

1.30-2.20 *Plenary Talk 2:* Vivian Irish (Yale University)
Petal Development: variations on a theme

2.20-2.40 Nana Hou Liu* (University of Toronto, St. George)
The significance of floral organogenesis in *Pyracantha* M. Roem (*Rosacea*)

2.40-3.00 Anne-Gaelle Rolland-Lagan (University of Ottawa)
Pattern formation in growing structures: a quantitative approach to the analysis of leaf vein patterning

3.00-3.45 Coffee Break and **Posters 19-26** (Azrieli Theatre Lobby)

Session 4 Chair: Owen Rowland (Carleton University)

3.45-4.05 Arunika Gunawardena (Dalhousie University)
Ethylene and caspase-like proteases are involved in PCD-remodeling of lace plant leaves

4.05– 4.25 Ashley Marcellus* (Nipissing University)
Characterization of corm senescence patterns in *Eriophorum vaginatum*

4.25-4.45 Sonia Gazzarrini (University of Toronto, Scarborough)
Hormone cross talk during phase transitions in Arabidopsis

4.45-5.05 Rugang Li (ECORC, Agriculture and Agri-Food Canada)
Salicylic acid-induced transcription factor NtMYB1 regulates N gene mediated virus resistance

5.30-7.30 Wine and Cheese Social (Galleria, upper floor between University Centre and Tory Building)

*student presentations

Abstracts for the 41st Annual Plant Development Workshop

Oral Presentations:

PLENARY TALK 1

miRNA BIOGENESIS AND ROLES OF MIR172 IN FLOWER DEVELOPMENT

Xuemei Chen, Li Zhao[1], YunJu Kim, Sizolwenkosi Mlotshwa[2], Bin Yu, Liu Bi, and Theresa Dinh

Department of Botany and Plant Sciences, University of California, Riverside, CA 92521
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[2]Current address: Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208

microRNAs (miRNAs) are 21-22 nucleotide RNAs that regulate target genes in a sequence-specific manner. The pleiotropic developmental defects exhibited by mutants in miRNA biogenesis genes, such as *DICER-LIKE1* (*DCL1*), *HUA ENHANCER1* (*HEN1*), and *HYPONASTIC LEAVES1* (*HYL1*), indicate that miRNAs play crucial roles in plant development. The pleiotropic phenotypes of mutants in a gene named *DAWDLE* (*DDL*) led us to find that *DDL* is also a general player in miRNA biogenesis.
miR172 regulates *APETALA2* (*AP2*) and other target genes and acts in the control of flowering and floral patterning. We studied the role of miR172-mediated repression of *AP2* in floral patterning. miR172 acts independently of *AGAMOUS* (*AG*) to control the activity of floral stem cells. miR172 acts through *AG* to control the inner boundary of the domain of expression of *APETALA3* and *PISTILLATA*, which specify petal and stamen identities. Expression of miR172-resistant *AP2* in Arabidopsis and Nicotiana benthamiana resulted in similar floral phenotypes, suggesting that the role of miR172 in flower development is similar in the two species.

NATURAL VARIATION IN SHOOT MORPHOLOGY OF *A. THALIANA* CAUSED BY *HUA2*

Qing Wang[1], Uday Sajja[1], Sarah Rosloski[1], Tania Humphrey[1], Min Chul Kim[2,3], Kirsten Bomblies[2], Detlef Weigel[2], Vojislava Grbic[1]

[1]Department of Biology, University of Western Ontario, London, ON N6A 5B8, Canada
[2]Department of Molecular Biology, Max Planck Institute for Developmental Biology, D-72076 Tübingen, Germany;
[3] Department of Agronomy, College of Agriculture and Life Science, GyeongSang National University, 900 Gajwa-dong, Jinju 660-701, Korea

Differences in life history strategy are thought to contribute to adaptation to specific environmental conditions. Among life history traits in plants, flowering time and shoot morphology are particularly important for reproductive success. Even though flowering time and shoot morphology are linked, the evolutionary changes in the genetic circuitry that simultaneously affects both traits remain obscure. Here, we have identified changes in a putative pre-mRNA processing factor, *HUA2*, as being responsible for the distinct shoot morphology and flowering behavior in a natural strain of *Arabidopsis,* Sy-0. *HUA2* has previously been shown to positively regulate two MADS box genes affecting flowering time (*FLOWERING LOCUS C* [*FLC*]) and floral patterning (*AGAMOUS* [*AG*]) [Chen and Meyerowitz, 1999; Doyle et al., 2005]. We demonstrate that natural changes in HUA2 activity have opposite effects on its known functions, thus having implications for the coordinate control of induction and maintenance of floral fate. The changes in Sy-0 lead to enhanced *FLC* expression, resulting in an enlarged basal rosette and aerial rosettes, while suppression of *AG* function favors a reversion of floral meristems from determinate to indeterminate development. Natural variation in HUA2 activity thus coordinates changes in two important life history traits, flowering time and shoot morphology.

1. Chen, X., and Meyerowitz, E.M. (1999). *HUA1* and *HUA2* are two members of the floral homeotic *AGAMOUS* pathway. Mol. Cell 3, 349-360.
2. Doyle M.R., Bizzell, C.M., Keller, M.R., Michaels, S.D., Song, J., Noh, Y.S., and Amasino, R.M. (2005). *HUA2* is required for the expression of floral repressors in *Arabidopsis thaliana*. Plant J. 41, 376-385.

CHARACTERIZATION OF TCP8 AND TCP15 GENES IN *ARABIDOPSIS THALIANA*; GENES WITH IMPLICATIONS FOR REGULATING MULTIPLE ASPECTS OF PLANT ARCHITECTURE.

Rashida Patel[1], and Dan Riggs[1].

[1]Department of Cell & Systems Biology, University of Toronto, Ontario CANADA, M1C 1A4

TCP genes encode a novel class of plant specific transcription factors that contribute to governing a variety of aspects of plant development, including leaf curvature, cell proliferation, floral organ asymmetry, and lateral branching. Members of this family contain a conserved motif dubbed the TCP domain, which is responsible for DNA binding and regulation of target gene expression. This class of proteins is so named after the three founding members; *teosinte branched1* found in maize, *cycloidea* found in *Antirrhinum*, and the PCF proteins found in rice. Multiple members of this family exist in different plant species, with 24 TCP encoding genes present in the *Arabidopsis* genome. Previous work has shown the role of the hormones auxin and cytokinin in influencing multiple aspects of plant growth and development, such as root and shoot proliferation, and lateral organ initiation. As such, I have been exploring TCP gene activity in regulating specific aspects of plant development through hormone mediated signaling pathways.

TCP15 was identified in an enhancer trap library screen for lines exhibiting nodal expression patterns. A BLAST homology search revealed that this gene is similar to a cotton EST encoding an auxin induced basic helix-loop-helix transcription factor. Expression of TCP15 was examined using this enhancer trap line and found to have an intriguing pattern that mirrors that of the auxin reporter DR5::GUS. Preliminary results suggest that this gene is not directly modulated in expression by auxin, however its expression pattern is altered in the presence of the polar auxin transport inhibitor NPA. The effect of the hormone cytokinin was also examined using root elongation assays on wild type, TCP15 null and TCP15 overexpressing seedlings propagated on several concentrations of cytokinin enriched media. The overall effect was that these various mutants exhibit a retardation in the physiological response to cytokinin, as compared to wild type seedlings. Homozygous TCP15 null mutants had no apparent phenotype. The high degree of TCP gene redundancy in Arabidopsis may account for the lack of an observable phenotype in the single mutant.

TCP8 is a member of the same TCP subclade as TCP15, and is highly similar in sequence to TCP15. The loss of function mutant reveals a striking alteration in cauline leaf morphology such that leaves in the mutant exhibit extensive margin serration in addition to possessing a greater number of trichomes on the abaxial leaf surface compared to wild type. The development of serration formation and quantification of trichomes is being examined using scanning electron microscopy. Preliminary vasculature analysis has revealed a change in venation pattern and number between the null and wild type. The competency of rosette and cauline leaves to respond to hormonal (auxin and NPA) cues to pattern leaf margin shape was examined, and were found to be unaltered, thus TCP8 is functioning to pattern leaf margin development in an auxin and cytokinin independent pathway.

RALF, A SECRETED PEPTIDE INVOLVED IN PLANT REPRODUCTION, STOMATAL OPENING AND ROOT FORMATION.

Eric Chevalier[1] and Daniel P. Matton[1]

[1] Institut de Recherche en Biologie Végétale (IRBV), Université de Montréal, 4101 Sherbrooke Est, Québec, Canada, H1X 2B2.

Small secreted peptides have been shown to be involved in intercellular signaling in numerous physiological processes including self-incompatibility, pathogen recognition, cell differentiation and cell growth. Most have been shown to act through a receptor-mediated pathway, suggesting a common theme in the recognition process at the plasma membrane. The Rapid Alcalinization Factor, or RALF, is a 5 kDa peptide initially isolated from tobacco and showed to play a role in development by inhibiting root growth. It was found to interact with unknown membrane proteins, and this recognition step leads to the activation of a MAPK cascade, an alkalinisation of the extracellular medium and an increase of cytosolic Ca^{2+}. However, no genetic studies are available and no fine analysis of physiological action of RALF peptide was published. Using promoter :GUS reporter gene assays, we showed that *RALFL* genes are expressed in specific cells, like pollen and guard cells, and at the root tips. These results show that some members are highly tissue specific and that redundancy can occur between each members, which made hard the genetic mutants analysis. To assess the function of this peptide on these cells, *in vitro* experiment was tested on pollen germination, pollen elongation, stomatal opening and root elongation with a synthetic peptide. The inhibition of pollen and root elongation, like a potential effect on stomatal closure, correlate with the cellular response of RALF peptides. Using various RALF :GFP fusion proteins, we also show by fluorescence microscopy and western blotting that, following the initial signal peptide cleavage in the endoplasmic reticulum (ER), N-terminal processing of the proRALF peptide to the active RALF occurs downstream of the ER compartment.

SIGNALS IN THE UNDERGROUND: MICROBIAL SIGNALS AND PLANT DEVELOPMENT

Donald L. Smith

Plant Science Department, McGill University, Montreal, Quebec

Plants are the primary source of energy for the biosphere. Some of this is released to other elements of the biosphere while the plants are still alive, in some cases to organisms that benefit the plants. A plant growing in the field is not a single organism; it is a community. We have examined aspects of plant-microbe interactions for the past 20 years. Initial work demonstrated that low spring soil temperatures were slowed the development and establishment of the nitrogen fixation symbiosis on legumes, and that the greatest effect was through disruption of the initial signal exchange between the two symbiotic partners. When we added appropriate signals to the bacterial symbiont, prior to its inoculation onto the plants, the symbiosis developed more quickly. Flavonoid signals were found to be effective. Addition of flavonoids or jasmonates causes the bacterial symbionts (rhizobia) to produce nod factors (lipo-chitooligosaccharides), which alert the plant to the presence of the correct types of rhizobia. Interestingly, we found that nod factors themselves can cause direct acceleration of plant growth and development, and that this effect is not confined to legumes. We have also been working with a series of plant growth promoting rhizobacteria that are not symbiotic nitrogen fixers. We found that some of these stimulate the early growth and development of soybean plants, leading to accelerated nodulation and nitrogen fixation. We also found that when these bacteria were removed from the cultures that they were grown in the culture media retained the capacity to stimulate plant growth and development. We have now isolated several new microbe-to-plant signals from these media. These signals have proven to be proteins. In at least some cases we have also shown that these proteins are bacteriocins (narrow spectrum antibiotics, some of which have recently been commercialized as food preservatives). The published literature offers increasing information regarding signaling among bacteria and it now begins to seem that prokaryotes and eukaryotes also "chat" and control each others development and function.

PATCHY ENCODES A PUTATIVE BETA-XYLOSIDASE/ARABINOFURANOSIDASE REQUIRED FOR PECTIN SIDE-CHAIN MODIFICATION IN ARABIDOPSIS MUCILAGE SECRETORY CELLS (MSC)

Andrej A. Arsovski, Theodore M. Pompa, George W. Haughn, Nicholas C. Carpita, Maureen C. McCann and Tamara L. Western

Biology Department, McGill University, Montréal, QC, Canada

In *Arabidopsis*, the epidermal cells of the outer ovule integument differentiate through a complex process into specialized cells that produce mucilage between the primary cell wall and plasma membrane. Upon imbibition the mucilage expands rapidly, breaking through the primary cell wall and enveloping the seed. A mutation in *PATCHY* (*PTY*) causes a peculiar phenotype where mutants have patchy and delayed mucilage release compared with wild type seeds. These mutants appear to undergo normal mucilage production and mucilage secretory cell development. Cloning of *PTY* by plasmid rescue revealed a T-DNA insertion in *AtBXL1*, a gene encoding a beta-xylosidase/arabinofuranosidase. Molecular complementation, and two independent Salk T-DNA knockout lines in the same locus producing a similar 'patchy' release phenotype, confirms the gene is involved in mucilage release. Expression analysis with qualitative RT-PCR and a promoter GUS shows expression of *PTY* in all tissues tested, including 7, and 10 day old seeds, consistent with its proposed role in mucilage release and cell wall modification. Chemical analysis suggests that *pty* mutants have an increase in arabinose, specifically (1→5)-linked alpha-D-arabinofuranose in their seed coat mucilage when compared to wildtype. Genetic analysis suggests PTY may act independently of known MSC regulators.

FUNCTIONAL CHARACTERIZATION OF SMALL RAB-A1 GTPASES

Xingyun Qi, and Hugo Zheng.

Biology Department, McGill University, Montreal, Quebec, H3A 1B1

Plant cells contain an elaborate endomembrane system which is composed of the endoplasmic reticulum (ER), Golgi apparatus, vacuoles and plasma membrane (PM). These organelles are linked by highly regulated membrane trafficking, of which Rab GTPases are a family of important regulators. In mammalian and yeast cells, individual Rab GTPases are responsible for distinct vesicle-targeting events. In Arabidopsis, 57 putative Rab coding sequences have been identified. Genomic comparison indicated that there is a large elaboration in the Rab-A subfamily whereby 26 members can be grouped into 6 structural subclasses Rab-A1 to -A6. Rab-A proteins share high sequence similarity to mammalian Rab11 that is involved in membrane trafficking between recycling endosome and the PM. It has been suggested that the diversification in Rab-A GTPases might be required to meet plant specific membrane traffickings for the formation of structures such as the cell wall. For example, In Arabidopsis, Rab-A4b is localized to the tips of growing root hair cells and interacts with a lipid kinase during root hair morphogenesis. Tobacco Rab11b is also found in the apical clear zone of elongating pollen tubes and it is involved in the secretion of cell wall proteins. In this study, we focused on the functional analysis of the Rab-A1 subclass, which consists of 9 members. Screening of publicly available microarray data on bar.utoronto.ca[1] revealed that three of the *RAB-A1* genes have generalized transcription patterns with strong expression in certain tissues while four of them are pollen-specific, one is root-specific and one has the highest expression in old seeds. Using semi quatitative RT-PCR, we confirmed most of the expression patterns. However, some discrepancies were also found. Currently, we are characterizing T-DNA insertion lines for each Rab-A1. Native *RAB-A1* promoter-GUS fusions and GFP-RAB-A1 fusions are also in construction to study the transcription pattern of Rab-A1 members in detail and to figure out the cellular distribution and subcellular localization of Rab-A1 members. Finally, to overcome the possible function redundancy and lethality of Rab-A1s in T-DNA insertion lines, we are currently also constructing dominant negative mutant forms of RAB-A1s under the chemically inducible synthetic promoter pOp6 using the hormone dexamethasone.

1. Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Schölkopf, B., Weigel, D., and Lohmann, J. 2005. A gene expression map of *Arabidopsis thaliana* development. Nat. Genet. 37, 10.1038/ng1543.

PLENARY TALK 2

PETAL DEVELOPMENT: VARIATIONS ON A THEME

Vivian F. Irish

Yale University, Departments of Molecular, Cellular and Developmental Biology and of Ecology and Evolutionary Biology.

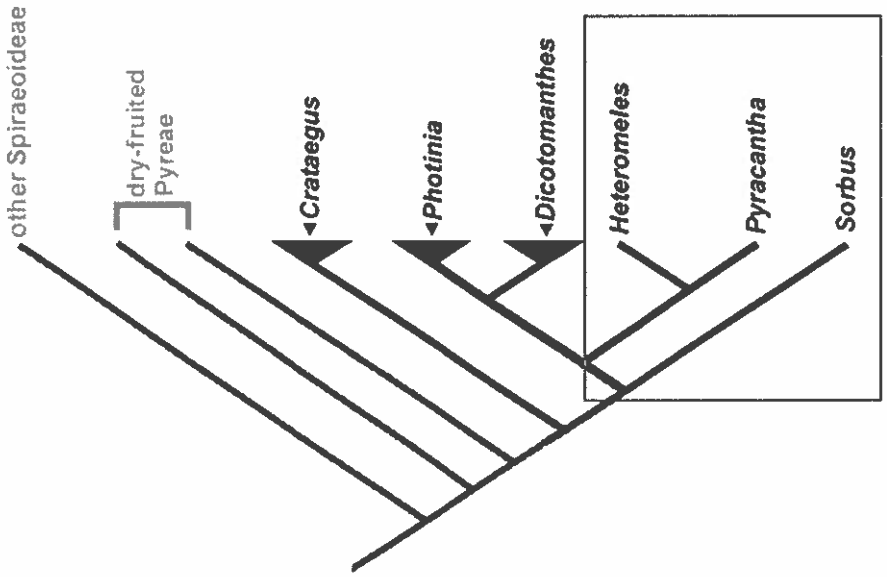
Nearly all angiosperm flowers possess a perianth, a series of sterile organs that surround the reproductive organs, the stamens and carpels. In many flowering plants, the perianth is differentiated into distinct outer organs, sepals, and inner organs, petals; this is termed a bipartite perianth. Our current understanding of how petal identity is specified rests largely on functional analyses carried out in several core eudicot species, including *Arabidopsis thaliana* and *Antirrhinum majus*. In these species, orthologs of the *APETALA3 (AP3)* and *PISTILLATA (PI)* MADS box genes are required to specify both petal and stamen identity. However, it is generally accepted that a bipartite perianth with distinct petals evolved independently multiple times within the flowering plants, leading to the possibility that homologous MADS box genes may be playing different roles in different flowering plant lineages. We have characterized the evolution of the *AP3* and *PI* MADS box gene lineages, and have found that numerous gene duplication and loss events have occurred throughout the history of these genes. To explore the consequences of these molecular changes on the specification of floral organ identity, we have carried out functional analyses in several core eudicot and basal eudicot species. Our results suggest that MADS box gene duplications have led to extensive functional diversification in specifying floral organ identities. Based on these observations, I will discuss a model for how the petal developmental program has evolved.

THE SIGNIFICANCE OF FLORAL ORGANOGENESIS IN *PYRACANTHA* M. ROEM. (ROSACEAE)

Nana Hou Liu

Department of Ecology and Evolutionary Biology, University of Toronto,
25 Wilcox Street, Toronto, ON, M5S 3B2

Rosaceae with hypanthial (= inferior) ovaries appear to have arisen from ancestors in subfamily Spiraeoideae with apocarpous gynoecia. Within Rosaceae tribe Pyrinae (included in the Spiraeoideae, and formerly known as subfamily Maloideae) two sister genera differ in gynoecium composition: flowers of *Pyracantha* have the hypanthial ovary typical of the tribe, whereas those of *Heteromeles* have apocarpous gynoecia. Gynoecial development in *Pyracantha* proceeds from simultaneous initiation of discrete gynoecial primordia rather than from the formation of a ring primordium as in *Heteromeles* and most other Pyrinae. The dorsal margins of the gynoecial primordia (either separate or ring-shaped) are continuous with the adjacent hypanthium. However, with the lower half of the ovary basally adnate to the hypanthium, the *Heteromeles* flower appeared more similar to the partially epigynous flower of *Pyracantha* than to the completely perigynous flower of *Dichotomanthes*. The contrast between *Pyracantha* and *Heteromeles* results from differences in the extent of intercalary growth below the hypanthium and gynoecium.



Tree based on results in Campbell, C. S., Evans, R. C., Morgan, D. R., Dickinson, T. A. & Arsenault, M. P. (2007). Phylogeny of subtribe Pyrinae (formerly the Maloideae, Rosaceae): limited resolution of a complex evolutionary history. *Pl. Syst. Evol.* 266: 119-145.

PATTERN FORMATION IN GROWING STRUCTURES: A QUANTITATIVE APPROACH TO THE ANALYSIS OF LEAF VEIN PATTERNING

Anne-Gaelle Rolland-Lagan

University of Ottawa, Canada K1N 6N5.

As an organism grows, different sets of genes are expressed in different regions and affect development. But what specifies domains of gene expression? How does the changing shape and size of an organism as it grows affect spatial and temporal patterns of gene expression? What controls shape as an organism develops? This is an exciting time for developmental biology, as recent advances in molecular biology, 2D and 3D imaging, and computer technology now make it possible to investigate these questions. The formation of leaf vasculature provides a good system for investigating the relationships between growth and patterning, as vein pattern formation and leaf growth are closely coupled, and the plant hormone auxin plays a major role in both processes. In the last few years, molecular work on auxin transport and proteins involved in auxin influx and efflux has sparked a renewed interest in theoretical models of vein pattern formation. However, the lack of quantitative data on vascular patterns during leaf ontogeny makes it difficult to assess models' validity.

We developed algorithms for analyzing vein pattern formation quantitatively in space and time during leaf development, and applied them to the analysis of vein pattern formation in the first rosette leaf of wild type *Arabidopsis thaliana*. In particular, we analyzed the size of vein network reticulations (loops), which enlarge and get split by new veins as a leaf develops. We found that leaf vein patterning can be divided into three phases: 1) at early developmental stages, veins form as the leaf enlarges, keeping vein density and loop areas homogeneous in time and space; 2) patterning then slows down relative to growth, and pattern heterogeneities in space start to appear; 3) at later stages, vein patterning has stopped and the pattern just enlarges due to growth. Temporal and spatial heterogeneities at later stages are therefore linked to growth heterogeneities. Differences in venation patterns between two compared groups of leaves may result from differences in patterning parameters, differences in the relative timing of the three phases of vein patterning, and/or differences in growth patterns. Growth patterns will have to be characterized and compared to venation patterns during leaf ontogeny to explore further how the two processes interact.

Quantitative analyses provide a way to compare leaf vein patterning phases and parameters in different species, different genotypes, and plants grown under different conditions. This should lead to new hypotheses on patterning mechanisms to incorporate in theoretical modeling studies. Quantitative data may also be extracted from simulation models, in order to compare experimental and theoretical data and test model validity. Quantitative analyses at the tissue level therefore provide a two-way link between theoretical and experimental work to further explore patterning mechanisms during leaf development.

ETHYLENE AND CASPASE-LIKE PROTEASES ARE INVOLVED IN PCD-REMODELING OF LACE PLANT LEAVES

L. Voicu[1], N.G. Dengler[1] and A.N. Gunawardena[2]

[1]Department of Cell and Systems Biology, University of Toronto, CANADA.
[2]Biology Department, Dalhousie University, CANADA.

The aquatic lace plant, *Aponogeton madagascariensis*, employs a unique mode of developmentally-regulated programmed cell death (PCD) that bears numerous similarities to apoptosis in animal cells. PCD remodels the leaf shape of lace plant, forming a lattice pattern of equidistantly-positioned perforations at a predictable time in leaf expansion. Therefore, this plant provides a highly tractable system for studying developmentally-regulated PCD.

In this system, distinct subpopulations of cells undergo cell death, whereas adjacent cells are unaffected and develop normally. Dying cells undergo a disruption of cytoplasmic streaming and loss of anthocyanin, indicators of tonoplast rupture, as well as DNA degradation and other cytoplasmic changes such as formation of vesicles, shrinkage and invagination of the membrane, and degradation of other organelles. Unlike many other forms of plant PCD, cell wall degradation is a key event in lace plant PCD and is required for formation of the open perforations in the leaf blade.

An ethylene biosynthesis inhibitor, L-α-(2-aminoethoxyvinyl) glycine (AVG), significantly suppressed perforation formation in leaves of axenically cultured lace plants, suggesting ethylene is an initial trigger of developmentally-regulated PCD in this system. Additionally, preliminary results using caspase-1 and -3 inhibitors support the possibility that caspase-like proteases may be involved in PCD during perforation formation in lace plant. The interconnection between ethylene and caspase-like proteases in developmentally-regulated PCD in plants is under current study.

CHARACTERIZATION OF CORM SENESCENCE PATTERNS IN *ERIOPHORUM VAGINATUM*

Ashley Marcellus and Ewa Cholewa
Department of Biology, Nipissing University

Eriophorum vaginatum is a tussock forming sedge that thrives in cold, nutrient poor environments. Its native habitats range from the peat-lands of the boreal forest to the wet sedge tundra of the Arctic. The perennial nature of *E. vaginatum* depends on the ability of the corm to store nutrients and survive winter. The corm senesces after flowering and seed production as its apical meristem undergoes change from being vegetative and producing leaves to becoming generative to produce inflorescence. Senescence refers to both the final step leading to the death of whole organism, and a complex, highly regulated process with crucial developmental function ongoing throughout the life of the plant. Because of the compacted growth pattern of *E. vaginatum* that causes the formation of an elevated tussock, it has been suggested that senescence of the individual corms progresses from the distal part of the corm. Cell vitality was determined by taking free-hand sections of the corm just below the apex, the mid-section and just above the distal part of the corm. Sections were stained with Evan's Blue, Neutral Red, and Fluorescein, than observed with a light and LCSM. In the sections from the apical part of the corm, Evan's Blue was retained in the cells indicating that the cells are undergoing senescence. However, samples from the mid and distal parts of the corm had little to no retention of Even's Blue, which indicate that cells in the mid and distal parts of the corm are still viable. This result was confirmed with Neutral red and Fluorescein. Senescence of the individual corms is initiated at the apex and progresses downwards. Therefore, the elevated habit of the *E. vaginatum* tussock is due to the progressive growth of new corms, not due to the senescence of the distal parts.

HORMONE CROSS TALK DURING PHASE TRANSITIONS IN ARABIDOPSIS..

Mimmie Lu[1], Joelle Dela Paz[1], Aathi Pathmanathan[1], Allen Tsai[1] and Sonia Gazzarrini[1].

[1] Department of Life Sciences, University of Toronto at Scarborough, 1265 Military Trail, Toronto, Ontario, Canada, M1C 1A4

The incredible variation observed in plant morphology often derives from changes in the timing of expression of certain developmental programs. During their lifetime, plants undergo major developmental phase transitions, such as embryonic-to-vegetative (germination) and vegetative-to-reproductive (flowering), both of which have been shown to be regulated by hormones. Mutational analysis has identified genes that accelerate or delay the timing of transition from the embryonic to the vegetative phase of development. The B3 domain transcription factor FUS3, which plays an important role in the establishment of late embryo functions, controls developmental phase transitions and foliar organ identity by regulating the levels of the hormones ABA and GA. Ectopic expression of FUS3 during vegetative development delays the embryonic-to-vegetative phase change and converts vegetative leaves into embryonic-like structures. Data will be presented on possible mechanism(s) that regulate FUS3 stability and turnover by hormones. Our data indicate that the regulation of FUS3 stability is mediated by a PEST motif located at its C-terminus. PEST sequences have previously been shown to induce protein degradation in plants and insects. Removal of the PEST motif in FUS3 is sufficient to increase FUS3 protein stability when expressed in plants and yeast. We also show that fusion of the FUS3 PEST motif is sufficient to induce GFP degradation and necessary for FUS3 sensitivity to hormones. A yeast two-hybrid approach is being used to identify proteins interacting with FUS3 and possibly involved in FUS3 degradation during early stages of seed germination.

SALICYLIC ACID-INDUCED TRANSCRIPTION FACTOR NTMYB1 REGULATES *N* GENE-MEDIATED VIRUS RESISTANCE AND PHENYLALANINE AMMONIA LYASE GENE EXPRESSION IN TOBACCO

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Plant *myb* genes are known to encode transcription factors regulating the phenylpropanoid biosynthesis, cellular morphogenesis, hormone-responsive pathways, and environmental stresses. However, their roles in plant defense response are not clear. We have previously isolated tobacco *myb1* (*Ntmyb1*) gene that was induced by tobacco mosaic virus (TMV) infection immediately preceding the *N* resistance gene-mediated hypersensitive response (HR) and systemic acquired resistance (SAR). To further elucidate the role of *myb1* in disease resistance, the *Ntmyb1* cDNA was placed under the control of CaMV *35S* promoter and introduced into tobacco cultivar (*Nicotiana tabacum* cv. Xanthi nc) carrying the *N* resistance gene. Enhanced resistance was observed in both T0 and T1 generation of transgenic plants as indicated by reduced lesion numbers, size and TMV accumulation. Cosegregation analyses of transgenic progeny showed that *Ntmyb1*overexpression led to enhanced TMV resistance, and that *Ntmyb1* gene silencing resulted in reduced TMV resistance. In contrast, progeny that lost *Ntmyb1* transgene exhibited wild type levels of resistance. *Ntmyb1* overexpression did not activate known pathogenesis-related PR genes but enhanced phenylalanine ammonia lyase (PAL) gene expression. Recombinant NtMYB1 protein was able to specifically bind to region of TMV-inducible PAL gene promoter. *Ntmyb1* overexpression in tobacco cultivar (*N. tabacum* cv. Xanthi nn) that lacks the *N* gene did not affect the appearance or severity of mosaic disease symptoms. Together, these results suggest that NtMYB1 regulates PAL gene expression and is involved in *N* gene-mediated virus resistance in tobacco plants.

Poster Presentations:

*student poster presentations

P1*
REGULATION OF FUSCA3 STABILITY BY THE HORMONES ABA AND GA.
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FUSCA3 (FUS3) is a key transcriptional regulator directing late embryogenesis. It promotes seed dormancy and acquisition of desiccation tolerance by increasing abscisic acid (ABA) level and down-regulating gibberrelic acid (GA) biosynthesis in the embryo. As a feedback mechanism, FUS3 protein level is destabilized by GA, while stabilized by ABA. We examined the possible mechanism by which FUS3 turnover is regulated by ABA and GA. Here we report that FUS3 degradation is dependent on the presence of a putative PEST motif present at its C-terminus. Removal of the PEST motif affects FUS3 function, stability and sensitivity to GA and ABA. We also show that the PEST motif is sufficient to induce protein degradation when fused to GFP. Our data suggest that FUS3 turnover and sensitivity to hormones is dependent on a PEST domain located at its terminal region and it is likely to involve protein turnover through the 26S proteasome.

P2*
ROOT MORPHOLOGY COMPARATIVE STUDY OF COMMON BEAN (*PHASEOLUS VULGARIS*) AND GREEN GRAM (*VIGNA RADIATA* L. WILCZEK): IMPLICATIONS FOR DRUG TOLERANCE
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Few studies related to drought tolerance have systematically looked at below-ground biomass, because of the difficulties associated with observing roots *in situ*. Yet, root development and architecture are probably involved in some of the most important components of plant response to water stress (Sponchaido et al., 1989). Several studies have shown that more drought-tolerant cultivars tend to demonstrate a greater root-to-shoot ratio, and deeper root profiles (Matsui and Singh, 2002; Rao, 2001; Grzesiak et al., 1997 for example). The objective of this experiment was to examine the early root development of two legume crops (common bean *Phaseolus vulgaris* and green gram, *Vigna radiata* L. Wilczek) that have previously shown different responses to water stress. In particular, green gram had shown an ability to maintain yields under moderate and severe water stress in field conditions (Bourgault et al., unpublished). Experience working with plants indoors hinted towards different rooting characteristics involved in a possible genetically-inherited mechanism for greater tolerance to water stress. Plants were grown in columns filled with sand in a greenhouse controlled environment with a 16 h photoperiod and temperatures of 28°C/20°C day/night times for about 20 days. Roots were harvested every 3-4 days by carefully washing down the sand from the bottom of the columns, then scanned and analysed with the WinRHIZO

system (Regent instruments, Ste-Foy, Canada). Preliminary results show that green gram indeed demonstrates a higher root length, thinner roots, and a deeper root profile than common bean, and this very early in its development. Meanwhile, its above-ground development is slower than common bean, suggesting a drought-avoidance strategy consisting in allocating a considerable portion of fixed carbon to roots. Thinner roots also seem to suggest a greater inherent efficiency in terms of volume explored per unit of allocated carbon. In addition, the scanning and analysis of roots with the WinRHIZO system provides a relatively fast and efficient way to observe a number of rooting systems. The authors suggest that screening for early root development using the approach described above could be an easy option as part of a greater strategy for the improvement of legume crops to drought tolerance.

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P3*

PROTEIN TARGETING WITH THE PLANT SECRETORY SYSTEM: ARE VACUOLES THE BEST PLACE TO STORE FOREIGN PROTEINS?

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The genetic modification of soybean plants to produce high levels of foreign bioproducts within the low value seed coat tissue has the potential to increase the value of soybean crops. To develop a successful system for this purpose, high levels of protein expression within the target tissue must be achieved. One means of increasing the yield of a foreign protein is through targeting that protein to a specific subcellular compartment. This can be done through the addition of N- and/or C-terminal propeptides that then direct the foreign protein to the desired compartment. Using the model plant *Arabidopsis thaliana*, the effect of targeting on the yield and stability of foreign proteins was investigated. The peroxidase activity in leaves of transgenic plants expressing a soybean-peroxidase-green fluorescent protein fusion, targeted to the apoplast, endoplasmic reticulum, or vacuole, was compared to transgenic plant expressing the non-targeted fusion protein. Targeting significantly improved total plant peroxidase activity, with the highest mean activity found to result from vacuolar targeting.

P4*

A REVERSE GENETICS APPROACH TO UNDERSTANDING MUCILAGE SYNTHESIS IN THE *ARABIDOPSIS* SEED COAT

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In *Arabidopsis*, differentiation of the epidermal cells of the seed coat after fertilization leads to the production of a pectinaceous mucilage, which is synthesized in the Golgi apparatus and secreted between the plasma membrane and the primary cell wall. Very little is known about which genes are involved in the synthesis of pectins. We are using two reverse genetics approaches to identify genes that may be involved in the synthesis of mucilage in the *Arabidopsis* seed coat. The first is datamining the expression patterns of predicted cell wall genes using publically available expression databases (Schmid et al., 2005) and expression visualization tools (Toufighi et al., 2005). The second approach is a microarray experiment performed between wild type seed and siliques and those of a known mucilage secretory cell developmental mutants. Putative knockout mutants for genes which showed high expression levels in wild type seed and silique tissues during peak mucilage synthesis were selected. To date, none have shown abnormal mucilage synthesis or seed coat development, suggesting perhaps genetic redundancy. Double mutants are being constructed and analyzed to further investigate these genes. RT-PCR is being used to confirm the expression results from the microarray data and to confirm that the mutant lines are true expression knockout mutants. Through the examination of these genes we hope to refine the proposed genetic pathway for the regulation of mucilage production during seed coat epidermal cell differentiation.

P5

GENETIC ANALYSIS OF ARABIDOPSIS-SPIDERMITE INTERACTION

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In response to herbivore attack, plants have evolved a variety of mechanisms to deter herbivore feeding, which prevent the herbivores from jeopardizing the plant's health, reproduction, and ultimately survival. The two-spotted spider mite *Tetranychus urticae* is a generalist herbivore that sucks out the plant cell's contents leaving small light-coloured punctures or spots on their leaves. Furthermore, the spider mite is a newly developed model organism that will, in the near future, provide tools for molecular analysis of its development, physiology and metabolism. The main objective of my research is to characterize the differential resistance of Col and L*er* Arabidopsis accessions to spider mite damage. My aim is to characterize damage on Arabidopsis plants upon spider mite feeding, to determine the genetic bases of this differential response, and to map genes responsible for the variation in susceptibility between L*er* and Col. A genetic dissection of mite-plant interactions could thus provide insights into the signalling and transcriptional basis of plant defences used against herbivores. In addition, genome-wide sequences of spider mites will lend themselves to the analysis of the

transcriptome's response to host-plant defensive compounds, providing an opportunity for making an important contribution to our understanding of plant-herbivore interactions

P6

DISSECTION OF THE *AtMYB61* REGULATORY CIRCUIT BY CHEMICAL GENETICS.

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As sessile organisms, plants have evolved mechanisms that enable the developmental plasticity that allows them to respond to fluctuations in their environment. *Arabidopsis thaliana* MYB61, a member of the R2R3-MYB family of transcription factors, is involved in the regulation of phenotypic plasticity in response to environmental cues, such as light and sucrose availability. *AtMYB61* regulates several key aspects of plant growth and development, including vascular architecture, secondary root formation, stomatal aperture, and flowering time. Though *At*MYB61 has been shown to play an important role in the control of these traits, the components of the regulatory circuitry that reside upstream and downstream of this transcription factor remain to be elucidated. One method that can be used to dissect a regulatory circuit is to disrupt the pathway using small molecules, and then to relate this disruption to the action of genes in the circuit. This approach, known as chemical genetics, is particularly amenable to high throughput analysis, where thousands of different chemicals are screened with relative ease. Recently, we employed a chemical genetics approach involving a screen of over 2000 different compounds that differentiated between wild-type *A. thaliana* seedlings relative to seedlings containing a *myb61* loss-of-function mutation. Interestingly, five different chemicals belonging to the sulfonamide family of compounds had a deleterious effect on dark-mediated elongation growth in wild-type seedlings, while the *myb61* T-DNA insertion mutants were relatively unaffected by the presence of the sulfonamides. Here we report on the initial chemical screen, and the progress we have made toward using the screen to characterise additional components of the *At*MYB61 regulatory circuit. We propose that sulfonamide insensitivity found in the *myb61* knockout seedlings can be used as a starting point to dissect the molecular components involved in both the upstream regulation and downstream manifestation of *At*MYB61 activity.

P7

A CENTRAL REGULATOR OF THE PLANT TRANSPIRATION STREAM.

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Plant transpiration is a major determinant of both global carbon fixation and terrestrial water cycles. Transpiration makes water available for photosynthesis and determines the rate of water loss via evaporation from leaves. Transpiration is largely influenced by the dynamic regulation of stomata, the formation of xylem, and root system architecture. Previously we showed that the R2R3-MYB transcription factor, *At*MYB61, was both necessary and sufficient to control the diurnal changes in the stomatal aperture. Here

we show that this same transcription factor directly controls the other major facets of the plant transpiration stream, xylem formation and root system architecture. *AtMYB61* is expressed in differentiating xylem cells and emerging lateral root primordia. Loss of *AtMYB61* function results in a decrease in xylem formation, alterations in xylem cell structure, and a decrease in lateral root formation. *AtMYB61* expression in differentiating xylem is contingent on the availability of photosynthate, providing a link between the establishment of the transpiration stream and photosynthesis itself. *At*MYB61 regulates a suite of downstream target genes, which in turn determine the extent and features of xylem cell formation and root system architecture. Thus, *At*MYB61 is the first transcription factor that defines a genetic module directly linking the three major components of the plant transpiration control system, a key innovation in the evolution of terrestrial life.

P8

TRANSCRIPTIONAL REGULATION OF *AtMYB61* EXPRESSION BY NUTRITIONAL CUES. Christian Dubos[1], Cecile Vriet[1] and Malcolm M. Campbell[1,2]

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The transcription factor *At*MYB61 plays important roles in controlling facets of the plant transpiration stream. Here we show that *AtMYB61* is regulated in a novel manner by nutritional stimuli, including sugars and amino acids. Diurnal changes in *AtMYB61* transcript abundance are dampened in mutants impaired in diel carbohydrate cycling. Pharmacological analyses suggest that *AtMYB61* transcript abundance is influenced by soluble sugars, through a pathway that appear to require sugar metabolism, but does not directly involve hexokinase signalling. This hypothesis is supported by the finding that *AtMYB61* expression remains sugar responsive in the hexokinase (*HXK1*) loss-of-function mutant *gin2*. *AtMYB61* transcript abundance increases to the greatest extent in response to the major product of photosynthesis, sucrose, and is repressed in response to two major products of photorespiration, glutamate and glycine. *AtMYB61* expression is de-repressed by soluble sugars in a mechanism that involves intragenic sequences. Phylogenetic footprinting, bioinformatic, and biochemical analyses suggest a role for intron sequences in the regulation of *AtMYB61* expression. Based on these and other data, we present a model that explains the diurnal de-repression of *AtMYB61* expression by diurnal fluctuations in nutritional cues.

P9*

IDENTIFICATION AND CHARACTERIZATION OF GENES INVOLVED IN THE BIOGENESIS OF SMALL RNAS IN RICE, *ORYZA SATIVA* L.

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Small RNAs are now recognized to play a major role in the regulation of plant developmental processes. This research used a reverse genetics approach to investigate the role(s) of genes involved in small RNA biogenesis in rice, *Oryza sativa L.* Specific primers designed against rice orthologues of Arabidopsis genes involved in small RNA biogenesis were used to screen a ethylmethanesulfonate-mutagenized rice population using a TILLING approach. A mutant containing a single nucleotide polymorphism (SNP) in the intronic region of the gene silencer of gene silencing 3 (*Ossgs3*) was identified. *Ossgs3* SNP homozygous mutants showed abnormal leaf curling, which segregated according in a Mendelian fashion. A T-DNA knockout of this gene was received from the Korean Postech Plant Functional Genomics Laboratory. Morphological analysis of the *Ossgs3* T-DNA mutant suggests that there may be increased fiber deposition in these plants. Our preliminary results suggest that OsSGS3 is involved in the production of ta-siRNA which targets the transcription factor, Auxin Response Factor (AFR) for degradation. In the *Ossgs3* SNP and T-DNA mutants, the increase in ARF leads to an increased sensitivity to the phytohormone auxin which results in changes to leaf morphology.

P10*

SEQUENCING FOUR PROSPECTIVE HOUSEKEEPING GENES FROM PULSE CROPS TO ENABLE VALIDATION OF THEIR USE IN NORMALIZING FUTURE REAL-TIME REVERSE TRANSCRIPTION PCR ASSAYS IN ADDITION TO OPTIMIZING GROWTH CONDITIONS REQUIRED FOR RAPID REPRODUCTION OF THE PULSE CROPS UNDER STUDY.

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The use of real-time reverse transcription PCR (rtPCR) is a powerful tool for the analysis of gene expression. However, to ensure accuracy, it is vital to normalize data, such as with the use of internal reference (or "housekeeping") gene. Several potential housekeeping genes have been identified for use in plant studies, including ribosomal protein L2 and actin. Since no sequence for these genes were available in the target pulse crops (chickpea, field pea and lentil), cDNA based cloning using degenerate primers was employed. The resulting sequences obtained for ribosomal protein L2 and actin showed 65-93% and 78-96% sequence identity, respectively, when compared with sequences for these genes from closely related plants. It is also important to maintain plants in a controlled and reproducible environment when undertaking gene expression

studies. As such, the determination of optimal growth chamber conditions for the production of mature plants is critical. This study found that plants grown under intense light and long day conditions and supplemented Hoagland's nutrient solution were the most successful.

P11*

CLONING AND TISSUE EXPRESSION ANALYSIS OF CYSTATHIONINE γ-SYNTHASE FROM CHICKPEA, FIELD PEA, AND LENTIL: TOWARDS AN INCREASED UNDERSTANDING OF THE METHIONINE BIOSYNTHETIC PATHWAY.

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Pulse crops (e.g. chickpeas, field peas, and lentils) are a major staple food for both humans and non-ruminant animals; however, they contain nutritionally insufficient levels of the required amino acid methionine. Previous attempts to raise methionine levels in plants, including the increased expression of genes of the methionine biosynthetic pathway and anti-sense constructs of genes from competing pathways, have met with limited success. These attempts demonstrate that a greater understanding of the regulation of these pathways is required to enable more subtle approaches for altering methionine levels. In plants, cystathionine-γ-synthase (CGS) catalyzes the committing step in the transsulfuration pathway, which produces the immediate precursor of methionine. The genes encoding CGS from chickpea, field pea and lentil have been cloned and sequenced. Analysis has revealed 81-92% sequence identity of the pulse crop genes with CGS from other plant species. Investigation of the tissue expression pattern of CGS in chickpea, field pea and lentil is underway.

P12*

INVOLVEMENT OF THE SOLAMUN CHACOENSE MAPKKK ScFRK1 IN EMBRYO SAC DEVELOPMENT.

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The *Solanum chacoense* Fertilization-Related Kinase 1 is a member of the MEKK subfamily of plant MAPKKK that is specifically expressed in ovules. RNA gel blot analysis showed that *ScFRK1* mRNA levels decrease rapidly following pollination. In situ hybridizations showed that *ScFRK1* mRNAs accumulate predominantly at the distal end (tip) of the growing integument during early ovule development. Later, *ScFRK1* expression is almost exclusively detected in the egg apparatus cells of the embryo sac. These results suggest both pre- and post-fertilization roles in ovule development. Transgenic plants down-regulated in *ScFRK1* expression showed no abnormal phenotypes in vegetative tissues but produced seedless fruits upon pollination. To define where and when during ovule development abnormalities appeared, cleared ovules from affected transgenic lines were observed by differential interference contrast. At anthesis, higher frequency of abnormal embryo sacs correlated with lower *ScFRK1* expression in these transgenic lines, confirming the link between *ScFRK1* expression and the

formation of a functional embryo sac. Using flower buds at various developmental stages it was possible to show that in plants weakly expressing the *ScFRK1* gene, formation of the female gametophyte did not progress further than the functional megaspore stage (FG1). No further divisions were observed, leading to the formation of ovules devoid of an embryo sac. Surprisingly, even in severely affected plants producing no embryo sac, pollination led to the production of parthenocarpic fruits. Using a semi *in vivo* pollen tube growth system *ScFRK1* mutant lines were also shown to be severely affected in pollen tube guidance, confirming the involvement of the egg apparatus cells as the source of attracting molecules. With this system, we also observed that the attractant signal is specie specific.

P13*

CHARACTERIZATION OF RALF PEPTIDES FROM SOLANUM CHACOENSE AND ARABIDOPSIS THALIANA: FUNCTION IN PLANT DEVELOPMENT AND BIOCHEMICAL PROPERTIES.

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Actually, there is an emergence of studies on small peptides which affect plant development. The systemins, phytosulfokines, SP11/SCR and CLAVATA3 are small peptides that act through a signaling pathway mediated by receptor kinase. Several others small peptides have properties that lead us to believe that they act through a signaling pathway. One of them is the *Rapid Alcalinization Factor* (RALF), a small peptide originating from a preproprotein. It was first identified in the leaves of *Nicotiana tabacum* (NtRALF) concomitantly with systemins. In our *Solanum chacoense* ovary EST database, five RALF-like genes, named ScRALF1-5, were identified using homology search using the NtRALF amino acid sequence. ScRALF1 and ScRALF3 display an ovary specific expression while ScRALF2, ScRALF4 and ScRALF5 show ubiquitous expression. Could some RALFs play a role in fruit development? RNAi plants for ScRALF1 and ScRALF3 show large defect in the plant development: rounded leave, delayed flowering time, small flowers, small fruits and small stature are observed. Analyse of T-DNA insertional mutants for RALF-like genes in *Arabidopsis thaliana* are in progress and this will provide more insights about the function of RALF peptides. Moreover, GFP fusion proteins are made to determine the subcellular localization of RALF peptides and to gain more details about their post-translational processing.

P14*

THE SEED COAT OF *ARABIDOPSIS THALIANA* AS A MODEL SYSTEM TO STUDY PLASMA MEMBRANE TO CELL WALL ADHESION.

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Traditionally, plant cells are thought of as static and constrained because of their rigid cell walls. However, new evidence suggests a model in which plasma membrane and cell wall interactions are dynamic. As a result, there is considerable interest in

determining the key proteins that act to link the plasma membrane and the cell wall. While there are many hypotheses as to which gene families are involved in adhesion, few proteins have been directly implicated in this process.

The goal of my work is to use the seed coat of *Arabidopsis thaliana* as a model system to study plasma membrane to cell wall adhesion. Plasma membrane to cell wall interactions have been characterized in wild type seed coat cells using light and electron microscopy. In addition, plasmolysis studies were employed to specifically understand plasma membrane to cell wall adhesion in wild type seed coat cells.

Having characterized wild type plasma membrane to cell wall adhesion in seed coat cells, a reverse genetics approach was employed in order to determine key genes that are involved in this process. Twenty-two candidate genes were identified using publicly available gene expression profiles and data from seed-specific microarrays that were recently performed in the lab. Homozygous mutants were isolated and confirmed for all of these genes. Mutant plants were screened for defects in plasma membrane to cell wall adhesion via plasmolysis. Mutants were also screened for changes in seed coat morphology that may result form defects in adhesion. Preliminary results indicate that an arabinogalactan protein (AGP) and a fasciclin-like AGP may be involved in this process. Currently, further phenotypic analysis is being performed on these potential plasma membrane to cell wall adhesion mutants and double mutants are being produced and analyzed.

P15*

GENE EXPRESSION PATTERNS OF ALCOHOL-FORMING FATTY ACYL-COA REDUCTASES FROM *ARABIDOPSIS THALIANA*.

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We are characterizing an eight-member family of alcohol-forming fatty acyl-CoA reductases (FARs) from *Arabidopsis thaliana.* One of these genes, *CER4* (*At4g33790*), is responsible for the production of very-long-chain fatty alcohols present in the waxy cuticle that coats the aerial surfaces of Arabidopsis (1). We found that this gene is also expressed in all of the cell layers of the elongation zone of young roots. Three other Arabidopsis FAR genes, *At3g44540*, *At3g44550*, and *At5g22500*, were found to be expressed in root tissues by inspection of DNA microarray data. Using promoter-reporter gene fusions, we found that these three genes are specifically expressed in the cell layer surrounding the central vasculature of the root. Each of these genes had a specific developmental gene expression pattern, relating, for example, to the synthesis of lateral roots. Suberin and associated waxes (e.g. fatty alcohols) form a protective hydrophobic barrier that limits water and solute loss from the central stele of roots and also from the seed coat. Thus, these enzymes are likely responsible for the production of fatty alcohols associated with suberin. Analysis of microarray data indicated that these three genes are expressed in the seed and it is likely that they are expressed in the seed coat as this layer is the site of suberin deposition. *Atg5g22500* is also expressed in various aerial tissues, such as leaves and flowers. We have identified knockout mutations in each of these genes. We anticipate that the chemical phenotypes in the roots and seed coats of these mutants will reveal important roles for fatty alcohols in these specific cell types. The substrate specificities of the FAR enzymes are being

investigated by measuring the accumulation of fatty alcohols in yeast heterologously expressing FAR enzymes (2). We expect that this study will provide valuable information for producing drought-tolerant plants and for using the plant seed coat as a platform for the synthesis of high-value bio-products.

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P16*

CHARACTERIZATION OF THE SUBSTRATE SPECIFICITES OF TWO ALCOHOL-FORMING FATTY-ACYL REDUCTASES FROM ARABIDOPSIS BY EXPRESSION IN YEAST.

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Fatty acyl CoA-reductases (FARs) are enzymes involved in the synthesis of long chain and very-long-chain fatty acyl primary alcohols, which are found throughout the biological world in two forms; as free alcohols or in a combined state such as wax esters. Fatty acyl CoA-reductases (FARs) use NAD(P)H as a cofactor to convert a fatty acyl-CoA into a primary fatty alcohol and CoASH, via an aldehyde intermediate that is not released from the enzyme (1). A search of the *Arabidopsis* genome in publically available databases revealed a family of eight FAR-like proteins in the *Arabidopsis* genome that contain 28% to 54% amino acid identity to the amino acid sequence of a seed-specific FAR from jojoba (2). CER4 (At4g33790) is an Arabidopsis FAR important for production of C24, C26, C28 and C30 fatty alcohols associated with cuticular wax (3). Here, our research has focused on identifying the substrate specificites of three alcohol-forming FARs (*At3g44550*, *At5g22500*, and *At3g44540*) that are expressed in roots in the endoderm layer surrounding the stele suggesting a role in the production of fatty alcohols present in suberin (4). Heterologous expression of two *Arabidopsis* alcohol-forming fatty acyl reductases (FARs) in yeast (*Saccharomyces cerevisiae)* revealed distinct overlapping substrate specificities. Heterologous expression of *At3g44550* in yeast resulted in accumulation of C16, C18, and C20 fatty alcohols while expression of *At5g22500* resulted in accumulation of C18, C20, and C22 fatty alcohols. This is consistent with roles in the production of C18-C22 fatty alcohols associated with suberin. Characterization of the At3g44540 substrate specificity remains to be investigated. Biochemical characterization of this set of FAR enzymes is important as knowledge of the specificities of these enzymes has industrial applications, such as the high-level production of fatty alcohols of desired chain lengths in transgenic seed oils and microbes.

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P17*

BIO-IMAGING SURFACE WAX CRYSTALS OF *ARABIDOPSIS THALIANA* WAX-DEFICIENT MUTANTS USING SCANNING ELECTRON AND ATOMIC FORCE MICROSCOPY

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Epicuticular wax crystals are believed to play crucial roles in ecological functions, such as plant-pathogen interactions and in reflecting UV radiation. To date, approximately 30 wax-deficient mutant loci (termed *eceriferum* mutants) have been identified in the model plant *Arabidopsis thaliana*. We are using these mutants to identify and functionally characterize gene products involved in wax production and secretion. The objective of the present study was to perform phenotypic analysis of wax deficient *cer* mutants using scanning electron microscopy (SEM) and atomic force microscopy (AFM). More specifically, screening of stem wax crystals by SEM and AFM was used to explore plant surface morphology, composition and topography of known *cer* mutants and a set of uncharacterized lines in search of novel mutants. We have investigated by SEM the crystal structures on the stem surfaces of 11 known *cer* mutants and 10 uncharacterized mutants of Arabidopsis. The main crystalline structures observed were: platelets, dendrites, tubules, rods, and umbrellas. Further correlational studies of wax composition were performed using published literature (1). Dendrites correlated favorably with shorter carbon chain lengths (C24 and C26 aldehydes) while umbrella structures correlated best with long chain constituents (C30 primary alcohols and C31 secondary alcohols). Both rods and tubes correlated with C29 ketones and C29 secondary alcohols. However, even small variations in chemical constituents can alter the crystal shape (2), and thus firm correlations are difficult to establish. Previously unobserved perturbations of the wax crystalline forms were observed in a number of the previously uncharacterized mutants suggesting that they represent novel loci. The wax chemical profiles of these mutants will be further characterized by gas chromatography. Molecular identification of the genes corresponding to these mutant loci will provide important insights into wax biosynthetic pathways and provide tools for the genetic engineering of cuticular wax composition to generate drought and pathogen resistant plants. Atomic force microscopy (AFM) was used to reveal topographic properties of three extreme cases of surface wax density using wild-type (Landsberg *erecta* ecotype), *cer*1 and *cer*3, showing differences in surface roughness at the nano-meter scale.

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P18*

INTRODUCTION OF NITROGEN FIXING BACTERIUM *GLUCONACETOBACTER DIAZOTROPHICUS* INTO CORN

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Corn (*Zea mays*) is an important crop cultivated world wide. A major cost of corn production is the nitrogen fertilizer. One possible solution to provide low-cost nitrogen fertilizer in corn production is to introduce biological nitrogen fixation system in corn plant. *Gluconacetobacter diazotrophicus* is a free-living endophytic diazotroph which can fix nitrogen in sugarcane. In this study, we evaluated introduction of *G. diazotrophicus* into corn plant. Seventeen Canadian grain corn varieties and ten sweet corn varieties were inoculated with *G. diazotrophicus* strains PAL5 and *nifD::aph* mutant MAD3A. Colonization under different nitrogen fertilization conditions was evaluated. Plant sugar contents and total N were analyzed to determine their relationship with colonization and nitrogen fixation. *G. diazotrophicus* colonized nineteen corn varieties) after inoculation. The colonization efficiency varied among different corn varieties. The population of the bacterium ranged from 10 to 10^4 CFUs g^{-1} fresh tissue in different plants and in different types of tissues. Plant growth promotion was observed in some corn varieties. Nitrogen fixation by *G. diazotrophicus* is being studied in the laboratory and in fields with the management of N supply.

P19*

CROSS-TALK IN PLANT-INSECT INTERACTIONS. Mélanie Chapleau, Marie-Hélène Weech and Jacqueline C. Bede.

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In response to caterpillar herbivory, general defense responses are mediated through the octadecanoid phytohormones, such as jasmonic acid. However, some caterpillar species contain the enzyme glucose oxidase (GOX) in their saliva which may be a mechanism to interfere with these induced plant defenses. GOX catalyzes the oxidation of glucose to produce gluconate and hydrogen peroxide (H_2O_2). In plants, the signaling molecule H_2O_2 may be an upstream signal which may lead to the activation of pathways that interfere with JA- or ethylene-dependent pathways.

To tease out the roles of phytohormone pathways in mediating caterpillar-specific defense responses in plants, Arabidopsis mutants that are unable to mount an octadecanoid-dependent induced resistance (IR^-) or systemic acquire resistance (SAR^-) were subject to herbivory by caterpillars of the beet armyworm, *Spodoptera exigua*, that had intact or impaired salivary secretions. Gene and protein expression of antinutritive defenses, such as laccase (polyphenol oxidase) and trypsin inhibitor were evaluated. As expected, the IR^- mutant did not mount a defense response. However, wild type plants infested by caterpillars with intact salivary secretions showed lower levels of *Atlac* gene expression and trypsin inhibitor compared to plants eaten by caterpillars with impaired

salivary secretions. In SAR$^-$ plants, this difference between the two caterpillar populations was not observed.

P20

FRIABLE1 IS A PREVIOUSLY UNKNOWN MEDIATOR OF CELL ADHESION IN *ARABIDOPSIS*.

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We have identified an *Arabidopsis* mutant, *friable1* (*frb1*), which exhibits defective cell adhesion. The reduced cell adhesion in *frb1* mutants leads to cell dissociation, and sometimes even organ dissociation. Paradoxically, ectopic cell adhesion leading to organ fusion is also observed in *frb1* seedlings. Although these phenotypes are often seedling lethal, a proportion of seedlings recover and grow to adulthood. In contrast to other cell adhesion defective mutants isolated so far, there is no obvious reduction in the pectic fraction of isolated cell walls. Measurement of total cell wall sugars indicates that there is not a significant change in the monosaccharide composition of both cellulose and matrix components. In addition, using comprehensive microarray polymer profiling (CoMPP) we show that the majority of the pectic epitopes in *frb1* walls are unchanged compared to wild-type. We have cloned *FRB1* and it encodes a membrane bound, plant specific protein with no similarity to known proteins. The subcellular location of the FRB1 protein is predominantly in the Golgi apparatus, suggesting that its molecular function might be enzymatic.

P21*

DEVELOPMENT OF DIABETOGENIC GENES IN THE WHEAT ENDOSPERM TISSUE: GLOBULIN 1-LIKE GENES IN WHEAT OCCUR IN AT LEAST THREE COPIES.

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A wheat Globulin 1-like protein is linked to the development of Type 1 diabetes in rats and humans. A cDNA clone for a seed protein, WP5212, that is 80% homologous with wheat globulin 1 (Glb1) at the protein level, was identified from a wheat expression library by probing it with antibodies from diabetic rats (1). *Glb1* has been previously described as a single copy gene in maize and barley. The objective of the current project is to characterize *glb1* and its potential family members in *Triticum aestivum*, at the gene structure and chromosomal organization level, to better understand the structure, control, and evolution of this gene or gene family. We also want to determine the

location of Glb1 in a wheat seed. A better understanding of the nature of wheat *glb1* may allow us to assess the potential for invoking gene silencing in wheat, or screening for the existence of a naturally occurring *glb1*-negative wheat variety.

Wheat seed sections were hybridised with antibodies raised against Glb1 and the peanut allergen Ara HI. Immunofluorescence staining showed that Glb1 was expressed mainly in the aleurone layer.

A 459 bp fragment from *glb1* cDNA clone was used as a hybridization probe to screen the hexaploid wheat (*Triticum aestivum* L. 'Glenlea') BAC library from Agriculture and Agri-Food Canada to identify *glb1* family members. Screening resulted in 25 positive clones. Three unique sequences were found. These sequences share 99%, 90% and 85% identity to Glb1 at the amino acid level. Preliminary Southern blot analyses have indicated the presence of *glb1*-like sequences in all studied samples, representing hexaploid, tetraploid and diploid wheat species. Further characterization of the BAC clones and various wheat samples is underway.

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P22

INDUCTION OF PATHOGENESIS-RELATED PROTEINS DURING BIOCONTROL OF *RHIZOCTONIA SOLANI* WITH *PSEUDOMONAS AUREOFACIENS* IN SOYBEAN (*GLYCINE MAX* L. MERR.) PLANTS.

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To investigate the biocontrol effectiveness of the antibiotic producing bacterium, *Pseudomonas aureofaciens* 63–28 against the phytopathogen *Rhizoctonia solani* AG-4 on Petri plates and in soybean roots, growth response and induction of PR-proteins were estimated after inoculation with *P. aureofaciens* 63–28 (P), with *R. solani* AG-4 (R), or with *P. aureofaciens* 63–28 + *R. solani* AG-4 (P + R). *P. aureofaciens* 63–28 showed strong antifungal activity against *R. solani* AG-4 pathogens in Petri plates. Treatment with *P. aureofaciens* 63–28 alone increased the emergence rate, shoot fresh weight, shoot dry weight and root fresh weight at 7 days after inoculation, when compared to *R. solani* AG-4; P + R treatment showed similar effects. Peroxidase (POD) and β-1,3-glucanase activity of *P. aureofaciens* 63–28 treated roots increased by 41.1 and 49.9%, respectively, compared to control roots. POD was 26% greater in P + R treated roots than *R. solani treated roots. Two POD isozymes (59 and 27 kDa) were strongly induced in P + R treated roots. The apparent molecular weight of chitinase from treated roots, as determined through SDS-PAGE separation and comparison with standards, was about 29 kDa. Five* β-1,3-glucanase isozymes (80, 70, 50, 46 and 19 kDa) were observed in all treatments. These results suggest that inoculation of soybean plants with *P. aureofaciens* 63–28 elevates plant growth inhibition by *R. solani* AG-4 and activates PR-proteins, potentially through induction of systemic resistance mechanisms.

P23

LIPO-CHITOOLIGOSACCAHRIDES ENHANCE FLOWERING IN ANGIOSPERMS.

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Lipo chitooligosaccharides (LCOs) are signaling molecules that are involved in the initial stages of symbiosis between legumes and rhizobia. We have recently found that LCO from *Bradyrhizobium japonicum* (a microsymbiont of soybean) has the capacity to enhance flowering in a wide variety of plants. Application of Lipo-chitooligosaccharide Nod *Bj* V($C_{18:1}$, MeFuc) induced early flowering and number of flowers in a number of plant species including *Arabidopsis thaliana*, soybean (*Glycine max*), tomato (*Lycopersicun esculentuml*) and pea (*Pisum sativum*). Synthetic LCOs also showed similar results in induction of flowering in *Arabidopsis thaliana*. These findings seem to have potential application in crop production and in horticulture. The results may also aid in furthering the basic understanding of the biology of flowering as two of the above species are genetically tractable.

P24*

OVERLAPPING ROLES FOR BLADE-ON-PETIOLE1,2 AND ASYMMETRIC LEAVES1 IN REGULATION OF *BREVIPEDICILLUS*

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Spatial and temporal regulation of the class I homeobox gene *BREVIPEDICILLUS (BP)* determines multiple aspects of plant architecture, including pedicel structure, proximal-distal leaf patterning, leaf serration, and internode elongation. Misexpression of BP in leaves causes ectopic meristem activity on adaxial leaf surfaces and margins, leaf lobing, and shortened petioles. Similar phenotypes occur in *BLADE-ON-PETIOLE (BOP) 1* and *2* and *ASYMMETRIC LEAVES (AS) 1* and *2* mutants. a*s1* leaves are short, irregularly lobed, and lack a petiole whereas *bop1 bop2* leaves are elongated with leafy petioles. Both mutants display ectopic meristem activity in leaves and misexpress BP in leaf petioles (1-3). *BP* is expressed in inflorescences and the shoot apical meristem and functions in a complex with BELLRINGER (BLR), a BELL1-like homeodomain protein. Mutations in *bp* or *blr* cause defects in inflorescence architecture and phyllotaxis but do not affect meristem maintenance unless combined with other mutations (4, 5).

To understand how BOP1/2 and AS1 interact to regulate BP activity, we monitored *BP* expression in *as1*, *bop1 bop2,* and *bop1 bop2 as1* leaves using a BP::GUS reporter gene. Enhanced misexpression of BP was observed in the leaves of *bop1 bop2 as1* triple mutants indicating that BOP1/2 and AS1 contribute to different aspects of BP regulation. 35S:BOP1/2 plants displayed a range of *bp*- and/or *blr*-like phenotypes including shortened or irregular internodes, paraclades, and downward-pointing siliques indicating that BOP activity represses BP/BLR activity in meristems and inflorescences. Finally, *bop1 bop2* mutations partially suppressed the inflorescence phenotypes of *bp-1* and *blr* mutants, possibly through upregulation of compensating factors.

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P25*

CLONING AND ANALYSIS OF THE PROMOTER REGIONS OF GENES OF THE SULPHUR ASSIMILATION PATHWAY FROM *BRASSICA JUNCEA*

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Phytoremediation is a cost-efficient approach to cleaning up sites contaminated with heavy metals. It relies on plant species, such as *Brassica juncea*, with the ability to hyperaccumulate metals in their harvestable biomass. The balance of sulphur compounds, such as glutathione, in the cell likely plays an important role in the Cd tolerance of *Brassica juncea*. In the current study, ~ 4000 base pairs of the promoter regions of selected genes of the sulfur assimilation pathway have been cloned, including ATP sulfurylase, glutathione reductase, glutathione synthetase and γ-glutamyl-cysteine ligase. The promoter sequences were analyzed using the TFSearch, PlantCARE and PLACE databases to identify cis-acting regulatory elements and transcription factor binding sites. The results are compared to the corresponding promoter regions from the model plant species *Arabidopsis thaliana*.

P26*

CONTROL OF FLORAL-MERISTEM IDENTITY AND BRACT SUPPRESSION IN *ARABIDOPSIS THALIANA* BY *BLADE-ON-PETIOLE 1* AND *2*

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Arabidopsis plants produce a succession of leaves, inflorescences, and flowers throughout its life cycle. Each of these structures is derived from the shoot apical meristem (SAM), a small group of undifferentiated stem cells located at the shoot apex. During vegetative development, primordia with leaf identity form on the flanks of the SAM. During reproductive development, primordia with floral identity form on the flanks of the inflorescence meristem. Molecular and genetic studies have identified a number of genes whose activities are important for the promotion of floral meristem fate. These include *LEAFY* (*LFY*), *APETALA1* (*AP1*), and *UNUSUAL FLOWER ORGANS* (*UFO*), and *LATE-MERISTEM IDENTITY1* (*LMI1*). Here, we show that *BLADE-ON-PETIOLE1* and *2*, previously shown to control leaf and floral-organ patterning, also function in the promotion of floral fate. Leaf initiation in *bop1 bop2* double mutants occurs more slowly than in wild-type and the transition to flowering is delayed. Double mutants show an increase in the number secondary inflorescences and flowers develop with subtending bracts. A small percentage of flowers have an *ap1*-like branched architecture. To further examine these findings we made crosses to *lfy*, *ufo*, *ap1*, and *lmi1* mutants. Our results indicate that defects in inflorescence architecture and floral-meristem identity are synergistically enhanced in the resulting triple mutants. In *bop1 bop2 ap1-1* plants,

nearly all flowers are converted to highly branched flowering shoots and there is a marked increase in the size and number of floral bracts. Similarly, in *bop1 bop2 lfy-2* mutants there is a marked increase in bract formation and floral branching. In *bop1 bop2 lmi1-1* plants, bract formation is enhanced. These data indicate that BOP1/2 function as floral-meristem identity regulators. They promote floral fate and suppress bract formation in combination with other floral-meristem identity genes.

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